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Unique mode of acetylation of oligosaccharides in aqueous two-phase system by *Trichoderma reesei* acetyl esterase

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Abstract

Trichoderma reesei RUT C-30 acetyl esterase, known to catalyze transacetylation reactions in water/vinyl acetate two-phase mixtures, was studied with respect to regioselectivity of acetylation of oligosaccharides in aqueous environment. Using series of oligosaccharides and their methyl glycosides, it was found that the enzyme catalyzes an efficient acetylation at *O*-3 position of the non-reducing terminal units of gluco-, xylo- and manno-oligosaccharides and a less efficient acetylation of *O*-2 position of the reducing end units of gluco- and xylo-oligosaccharides. The axial hydroxyl group at *O*-2 position of the reducing end mannose in mannooligosaccharides was not recognized by the enzyme and its acetylation was not observed. The structure of isolated transacetylation products was established by NMR, ESI-MS analysis and on the basis on their resistance towards action of glycosidases acting from the non-reducing end of oligosaccharides. The position of acetylation allowed deduce on some of the structural requirements of the enzyme for the acetyl group acceptors. *T. reesei* RUT C-30 acetyl esterase was also found to be capable of liberation of acetyl groups from terminal units of oligosaccharides, which speaks for its classification as an exo-acting acetyl esterase.
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Keywords: Acetyl esterase; *Trichoderma reesei*; Transesterification; Oligosaccharides

1. Introduction

There is increasing demand for environmentally friendly processes yielding natural fibers and biodegradable polymers with modified hydrophobic and rheological properties. Enzymatic acylations of oligo- and polysaccharides and polyhydroxyl-compounds in general, could be more environmentally benign than classical chemical esterifications. Lipases and other enzymes such as proteases, are known to operate effectively in a reverse way to catalyze esterifications and transesterifications in organic solvents or low-water-content media [1–11], including specific acylations of low-molecular mass carbohydrates. In comparison with lipases and some plant esterases (wheat germ, orange peel) that have been used for decades as powerful tools for hydroxyl group protection and de-protection in carbohydrate chemistry [12], the catalytic potential of carbohydrate esterases, such as acetylxylan esterases and feruloyl esterases, is almost unknown beyond their physiological functions in plant cell wall degradation. Therefore hemicellulolytic esterases remain still relatively unexplored biocatalysts.

Known examples of acylation reactions onto carbohydrate acceptors catalyzed by unclassified enzymes presumably belonging to carbohydrate esterases include: transacetylation to Glc_2 and cellulose in aqueous medium in the presence of isopropenyl acetate catalyzed by an intracellular carboxylesterase from *Arthrobacter viscosus* [13] and acetylation of the amino groups of chitobiose and chitotetraose in an aqueous solution of 3 M sodium acetate using a chitin deacetylase from *Colletotrichum lindemuthianum* [14,15]. Acetylation of carbohydrates by AcXE from the wood-rotting fungus *Schizophyllum commune* (a member of carbohydrate esterase family 1) was found to proceed most efficiently in an *n*-hexane-vinyl acetate-sodium dioctylsulfosuccinate (DOSS)-water microemulsion [16]. β-1,4-Mannohexaose was the largest oligosaccharide that was successfully acetylated.

Abbreviations: Ac, acetyl group; AcE, acetyl esterase; AcXE, acetylxylan esterase; Glc_2–Glc_6, cellobiose–cellohexaose; Xyl_2–Xyl_6, 1,4-β-D-xylobiose–1,4-β-D-xylohexaose; Man_2–Man_5, 1,4-β-D-mannobiose–1,4-β-D-mannopentaose; $MeGlc_2$, methyl cellobioside; $MeXyl_2$, methyl 1,4-β-D-xylobioside; $MeXyl_3$, methyl 1,4-β-D-xylotrioside

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An interesting enzyme catalyzing efficient acetylation of carbohydrates has been found in the cellulolytic system of *Trichoderma reesei* RUT C-30 [17]. The enzyme appears to be identical with the oligosaccharide-deacetylating acetyl esterase described by Sundberg and Poutanen [18]. It was found to be capable of catalyzing acetyl transfer to carbohydrates not only in organic solvents, but also in water. The enzyme efficiently acetylates saccharides in two-phase system composed of aqueous solution of the acceptor and vinyl acetate as the acetyl group donor. Mono- and diacetyl derivatives of mono and oligosaccharides were formed in relatively high yields (50 to 80%) [17].

It was of interest to examine the potential of this enzyme to acetylate larger oligosaccharides, eventually polysaccharides. In this work we describe an interesting performance of the *T. reesei* acetyl esterase in the water–vinyl acetate two-phase system on three different series of oligosaccharides and their methyl glycosides as acetyl group acceptors. Quite unexpectedly the enzyme was found to acetylate almost exclusively the terminal glycopyranosyl residues in the oligosaccharides, more efficiently the non-reducing residue. Thus its physiological role seems to be deacetylation of terminal acetylated residues in oligosaccharides generated by the action of endoglycanases and exoglycanases or glycosidases. The mode of action of the investigated enzyme thus might correspond to an exo-deacetylating esterase operating only on non-reducing end and reducing end sugar residues on xylo-, gluco- and manno-oligosaccharides. The enzyme also differentiates between gluco and manno-configuration on the reducing end carbohydrate residue of the oligosaccharides.

2. Materials and methods

2.1. Chemicals

Cellobiose (Glc_2), methyl β-D-xylopyranoside, $MeGlc_2$ were from Serva. Xyl_2–Xyl_6 and Man_2–Man_6 were from Megazyme (Ireland). $MeXyl_2$ and $MeXyl_3$ were generous gifts from Dr. Pavol Kovac (NIH, Bethesda). Cellobiitol was prepared by reduction of Glc_2 with $NaBH_4$. Glc_3–Glc_6 were from Seikagaku Kogyo Corp., Japan. Orcinol was from Sigma. Vinyl acetate was from Merck.

2.2. Enzymes and activity determinations

Purified acetyl esterase of *T. reesei* RUT C-30 was isolated from extracellular proteins secreted by the fungus during its growth on cellulose by two step ion-exchange chromatography on CM- and DEAE-Sepharose [19]. Its specific activity was 85 U/mg of protein. The enzyme was free of enzyme activities hydrolyzing β-1,4-xylo-, cello- and β-1,4-mannooligosaccharides. The acetyl esterase activity was determined at room temperature on 4-nitrophenyl acetate as described by Johnson et al. [20]. One unit of the enzyme activity is defined as the amount liberating 1 μmol of 4-nitrophenol in 1 min. β-Xylosidase was a product of recombinant *Saccharomyces cerevisiae* strain [21], which was a generous gift from Prof. W.H. van Zyl (University of Stellenbosch, South Africa). Almond β-glucosidase was from Serva. Protein concentrations were determined using the assay of Bradford [22].

2.3. Thin-layer chromatography

Reaction mixtures were analyzed by TLC on aluminum sheets coated with Silica gel 60 (Merck, Germany) in the following solvent systems: S1, 1-butanol/ethanol/water (10:8:7, v/v); S2, 1-butanol/ethanol/water (10:8:6, v/v); S3, acetonitrile/water (8:2, v/v). Sugars were detected on dried chromatograms by orcinol–sulfuric acid reagent or by *N*-(1-naphthyl)ethylenediamine dihydrochloride reagent according to Bounias [23].

2.4. Enzymatic acetylation of oligosaccharides

Acetylation of oligosaccharides (6 mM) was carried out in water (200 μl) at a final acetyl esterase concentration $0.75\ U\ ml^{-1}$. The reaction was initiated by addition of 50 μl of vinyl acetate, followed by mixing and incubation at 25 or 30 °C in the dark without further stirring. The aqueous layers of the reaction mixtures were analyzed by TLC or frozen on dry ice after the removal of the upper vinyl acetate layer. The aqueous solutions were then lyophilized and analyzed by mass spectrometry.

Similar, larger scale incubation mixtures containing 30 to 300 mg of the oligosaccharides were used for preparation of acetylated derivatives to be characterized by NMR. Monoacetates of Glc_2, Glc_5 and Glc_6 were isolated from the reaction mixtures after 3-day incubations by chromatography on Silica gel in solvent systems S1 or S2 in 32, 41 and 79% yields, respectively. Preparation of cellobiose-diacetate (Glc_2-diAc) as the minor product of cellobiose acetylation (3% yield) required 7-day incubation.

Product of methyl α-D-mannopyranoside acetylation was isolated from a 3-day reaction mixture in 81% yield.

2.5. Treatment of acetylated oligosaccharides with glycosidases and acetyl esterase

Solutions of mono and di-acetates of Xyl_2 and Xyl_3 (20 mM) isolated as above were incubated with β-xylosidase (2 U/ml) at 30 °C. Aliquots were analyzed by TLC. Solutions of Xyl_2 and Xyl_3 incubated with β-xylosidase as positive controls were analyzed simultaneously.

Solutions of Glc_2-monoAc (20 mM) in 0.1 M sodium phosphate buffer (pH 5.5) were incubated at 30 °C with β-glucosidase (10 U/ml) and *T. reesei* acetyl esterase (1.5 U/ml), either separately or in combination, and analyzed by thin layer chromatography in the solvent system S1. Controls without enzymes and positive controls with non-acetylated oligosaccharides were run simultaneously.

2.6. NMR spectroscopy and mass spectrometry

The structure of the acetylated derivatives was deduced from ^{1}H, ^{13}C and/or $^{1}H/^{13}C$ 2D HSQC NMR spectra. ^{1}H and ^{13}C-

NMR spectroscopy were performed with a Bruker AM 300 spectrometer equipped with 5 mm probe at 300.13 and 75 MHz, respectively, using Me_4Si as internal standard.

Mass spectrometry was done on a LCQ DECA XP ion-trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA), using direct injection and electrospray positive mode ionization. Lyophilized reaction mixtures were dissolved in a mixture water–methanol (1:1) and injected. Major molecular ions *m*/*z* of non-acetylated acceptors, their mono- and di-*O*-acetylated derivatives, and their clusters were assigned.

3. Results and discussion

3.1. Acetylation of oligosaccharides and their derivatives modified at the reducing end

When series of different oligosaccharides were used as acetyl group acceptors for *T. reesei* acetyl esterase catalyzing transacetylation in water–vinyl acetate mixtures, it was found that β-1,4-xylooligosaccharides and β-1,4-glucosaccharides (cellooligosaccharides) were transformed to mainly mono-*O*-acetyl derivatives and in a lesser extent to di-*O*-acetyl-derivatives (Figs. 1 and 2). The number of the acetyl groups in the modified acceptors did not increase with their polymerization degree since only mono- and di-acetates were formed. Conversion to diacetates was much lover than to monoacetates in the case of cellooligosaccharides. Diffused spots of acetylated derivatives of xylooligosaccharides (Fig. 2) indicated the presence of several positional isomers formed due to acetyl group migration along the xylopyranoid ring [24]. This phenomenon was not observed with cellooligosaccharides, suggesting much higher stability of the primarily formed derivatives in comparison with acetates of xylooligosaccharides. When Xyl_2, Xyl_3 and Glc_2 were replaced by the corresponding β-methyl glycosides (Figs. 1 and 2), or when cellobiitol was used instead of Glc_2, only the formation of monoacetylated products was observed, suggesting that the second acetylation took place exclusively on the reducing end sugar residue. No reaction was observed in the water–vinyl acetate mixtures in the absence of the enzyme.

When β-1,4-mannooligosaccharides were used as acetyl acceptors, only formation of monoacetates was observed (Fig. 3). This interesting finding pointed to an important role of the configuration of the OH-group at position C-2 of the reducing end residue. Conversion to mannooligosaccharides monoacetates was lower than in the case of cello- and xylo-oligosaccharides and newly formed products exhibited only one sharp spot on TLC, indicating the presence of only one type of mono-Ac.

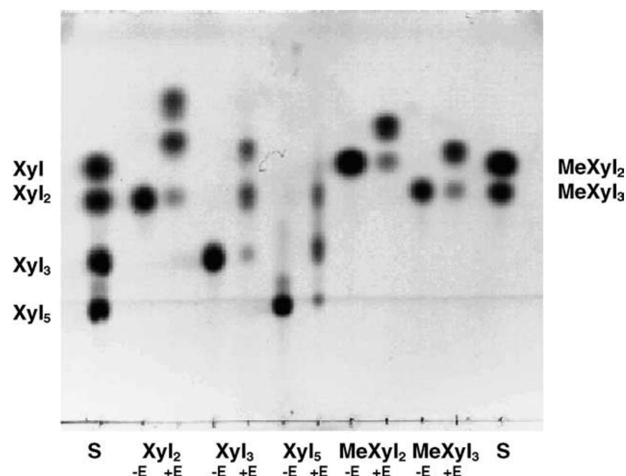

Fig. 2. Acetylation of xylooligosaccharides, $MeXyl_2$ and $MeXyl_3$ by acetyl esterase from *T. reesei* in water–vinyl acetate mixtures (+E) demonstrated by TLC and controls without enzyme (−E).

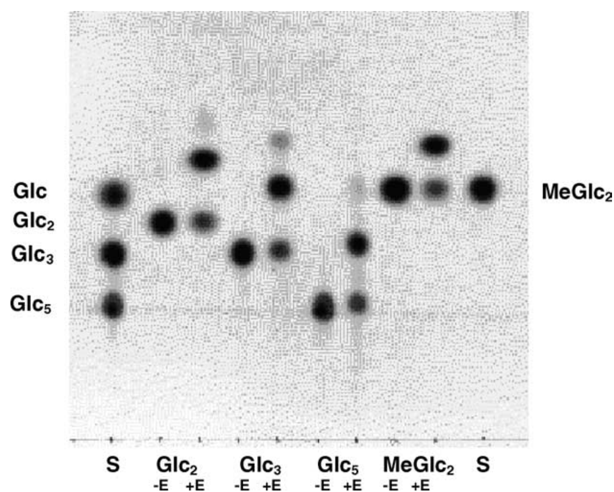

Fig. 1. Acetylation of cellooligosaccharides and $MeGlc_2$ by acetyl esterase from *T. reesei* in water–vinyl acetate mixtures (+E) demonstrated by TLC and controls without enzyme (−E).

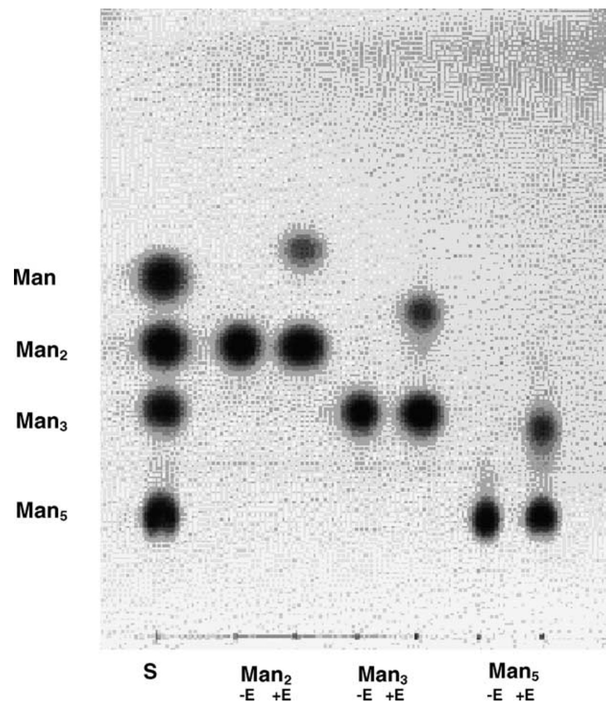

Fig. 3. Acetylation of mannooligosaccharides by acetyl esterase from *T. reesei* in water–vinyl acetate mixtures (+E) demonstrated by TLC and controls without enzyme (−E).

Table 1
Assignment of major ions obtained from ESI-MS of reaction mixtures of Xyl_2, Glc_5 and Man_5

Xyl_2		Glc_5		Man_5	
m/z	Ion type	*m/z*	Ion type	*m/z*	Ion type
389.2	$[Xyl_2Ac_2 + Na]^+$	851.5	$[Glc_5 + Na]^+$	851.4	$[Man_5 + Na]^+$
712.8	$[Xyl_2Ac + Xyl_2Ac_2 + Na]^+$	869.9	$[Glc_5AcH]^+$	852.4	$[Man_5 + Na]^+$
754.8	$[2(Xyl_2Ac_2) + Na]^+$	893.4	$[Glc_5Ac + Na]^+$	893.4	$[Man_5Ac + Na]^+$
755.9	$[2(Xyl_2Ac_2) + Na]^+$	894.4	$[Glc_5Ac + Na]^+$	894.4	$[Man_5Ac + Na]^+$
		935.4	$[Glc_5Ac_2 + Na]^+$	1721.3	$[Man_5 + Man_5Ac + Na]^+$
		936.4	$[Glc_5Ac_2 + Na]^+$	1763.4	$[2(Man_5Ac) + Na]^+$
		1325.6	$[3(Glc_5Ac) + 2Na]^{2+}$		
		1326.7	$[3(Glc_5Ac) + 2Na]^{2+}$		
		1346.5	$[2(Glc_5Ac) + Glc_5Ac_2 + 2Na]^{2+}$		
		1763.3	$[2(Glc_5Ac) + Na]^+$		
		1764.3	$[2(Glc_5Ac) + Na]^+$		
		1805.2	$[Glc_5Ac + Glc_5Ac_2 + Na]^+$		

Complete reaction mixtures of acetylation of cello-, xylo- and manno-oligosaccharides (similar to those presented at Figs. 1–3) were also analyzed by ESI-MS in the positive ionization mode. The ESI-MS data correlated well with the TLC analyses. The examples of assignment of major peaks in ESI-MS spectra of three selected reaction mixtures are shown in Table 1. The major peaks corresponded to sodium adducts of molecular ions of the starting oligosaccharides, their acetylated products and clusters of these molecules. Frequently, one Na^+ ion was also coupled to two molecules of the acceptor oligosaccharide and its acetyl derivatives, or of their combinations.

Some of the acetylated oligosaccharides, namely Glc_2, Xyl_2 and Xyl_3 mono- and di-acetates, and Glc_5 and Glc_6 monoacetates, were isolated and treated with β-glucosidase or β-xylosidase, the enzymes known to act from the non-reducing end units of gluco- or xylo-oligosaccharides [25–27]. Both glycosidases appeared to be inactive against any of the mono- and di-acetates. Under the same conditions they readily hydrolyzed non-acetylated counterparts. This served as evidence that one of the newly introduced acetyl groups was located at the non-reducing aldopyranosyl unit, thus preventing the action of β-glycosidases.

3.2. Positions of the acetyl groups in acetylated oligosaccharides and glycosides

The positions of the acetyl groups were investigated on acetylated products of Glc_2, Glc_5, Glc_6 and methyl α-D-mannopyranoside by NMR and enzymatic treatment with the corresponding glycosidases. The assignments of NMR signals were confronted with published data for compounds of similar structures [28–31].

The major compound of Glc_2 acetylation, the Glc_2-monoacetate was identified as 3-*O*-acetyl β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (3′-*O*-Ac-Glc_2, Table 2). In agreement with already published data [28,17], the introduction of an acetyl group in position *O*-3 of β-D-glucopyranosyl unit caused upfield shift of the C-3 resonance (1.6 ppm). The minor compound, a diacetate was identified as 3-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-(2-*O*-acetyl)-D-glucopyranose (Table 3). As in the previous case, one of the acetyl groups is bound at position *O*-3 of non-reducing glucopyranose residue, causing the same upfield shift of C-3 by 1.6 ppm. The position of the second acetyl group was assigned as 2-*O*-acetyl on the bases of the resonance shifts reported for mono-acylated D-glucopyranoses [28], causing 1.9 ppm upfield shifts for C-2 of

Table 2
^{13}C NMR data of 3-*O*-acetyl β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (Glc_2-monoAc, cellobiose monoacetate) and β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (Glc_2) (D_2O, 75 MHz)

Compound	Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6
Glc_2[a]	Reducing end α-anomer	92.6	72.2	72.3	79.6	71.0	60.9
Glc_2-monoAc		92.7	72.20	72.20	79.6	71.0	60.8
		(0)	(0)	(−0.1)	(0)	(0)	(−0.1)
Glc_2[a]	Reducing end β-anomer	96.7	75.2	74.8	79.5	75.7	61.0
Glc_2-monoAc		96.7	75.2	74.8	79.5	75.7	60.9
		(0)	(0)	(0)	(0)	(0)	(−0.1)
Glc_2[a]	Non-reducing end residue	103.5	74.1	76.4	70.4	76.9	61.5
Glc_2-monoAc		103.2	72.4	78.0	68.6	76.6	61.3
		(−0.3)	(−1.7)	(+1.6)	(−1.8)	(−0.3)	(−0.2)

The values in parentheses are the chemical shifts differences to the β-D-glucopyranosyl-(1 → 4)-D-glucopyranose. Positive differences indicate the upfield shifts.
[a] Data from Ref. [28].

Table 3
^{13}C NMR data of 3-*O*-acetyl β-D-glucopyranosyl-(1 → 4)-2-*O*-acetyl-D-glucopyranose (Glc_2-diAc, cellobiose diacetate) and β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (Glc_2) (D_2O, 75 MHz)

Compound	Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6
Glc_2[a]	Reducing end α-anomer	92.6	72.2	72.3	79.6	71.0	60.9
Glc_2-diAc		90.1	74.0	70.0	79.2	71.0	60.8
		(−2.5)	(+1.8)	(−2.3)	(−0.5)	(0)	(−0.1)
Glc_2[a]	Reducing end β-anomer	96.7	75.2	74.8	79.5	75.7	61.0
Glc_2-diAc		95.0	75.8	73.3	79.0	75.6	60.7
		(−1.7)	(+0.6)	(−1.5)	(−0.5)	(−0.1)	(−0.3)
Glc_2[a]	Non-reducing end residue	103.5	74.1	76.4	70.4	76.9	61.5
Glc_2-diAc		103.2	72.4	78.0	68.6	76.6	61.3
		(−0.3)	(−1.7)	(+1.6)	(−1.8)	(−0.3)	(−0.2)

The values in parentheses are the chemical shifts differences to the β-D-glucopyranosyl-(1 → 4)-D-glucopyranose. Positive differences indicate upfield shifts.
[a] Data from Ref. [28].

α-anomer and 0.6 ppm for C-2 of β-anomer. Both acetylated derivatives were resistant to the action of β-glucosidase. The *T. reesei* acetyl esterase deacetylated both Glc_2-acetates to Glc_2, and its application together with β-glucosidase resulted in the formation of free glucose.

Based on the above results one can conclude, that the main acetylation of Glc_2 takes place at position *O*-3 of the non-reducing end residue and the minor acetylation takes place at *O*-2 of the reducing end glucose. Although it is highly probable, that reaction mixture contained also β-D-glucopyranosyl-(1 → 4)-2-*O*-acetyl-D-glucopyranose (2-*O*-acetyl Glc_2), this derivative was neither detected nor isolated by the used techniques.

Similarly to Glc_2, the major product of Glc_5 acetylation was identified as 3-*O*-acetyl β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (Table 4). Compared to the literature data for Glc_5 [29,31] the acetyl group on *O*-3 of the non-reducing end glucopyranose residue caused 1.6 ppm resonance shift at the corresponding carbon. Glc_6-monoAc was prepared in the same way as Glc_5-monoAc. Similarly as in the case of Glc_5-monoAc, the presence of a single acetyl group in Glc_6-monoAc was proved by ESI-MS and by its resistance towards β-glucosidase action, confirming the acetylation of the non-reducing glucopyranosyl residue. Both Glc_5 and Glc_6 acetates were deesterified by the acetyl esterase, which was used for their preparation. Based on the obtained results and literature data [17] we can conclude that the acetyl group is also at *O*-3 position of the non-reducing end unit of cellohexaose (3-*O*-acetyl β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose).

The position of acetylation of the non-reducing mannopyranosyl residue in mannooligosaccharides was established indirectly. Readily available methyl α-D-mannopyranoside was used instead of expensive mannooligosaccharides as acetyl group acceptor. The glycoside was converted exclusively to a monoacetate. The position of the acetyl group at *O*-3 was confirmed by NMR spectroscopy, showing a typical upfield shift at C-3, caused by the introduced acetyl group [32]. Based on this result we propose that mannooligosaccharides are also acety-

Table 4
^{13}C NMR data of 3-*O*-acetyl β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (Glc_5-monoAc, cellopentaose monoacetate) and β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (Glc_5, cellopentaose) (D_2O, 75 MHz)

Compound	Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6
Glc_5[a]	Reducing end α-anomer	92.7			79.3		60.7
Glc_5-monoAc		92.8	72.2	72.2	79.4		60.8
		(0.1)			(0.1)		(0.1)
Glc_5[a]	Reducing end β-anomer	96.6	75.1	74.8	79.2	75.7	60.7
Glc_5-monoAc		96.7		74.9	79.2	75.7	60.8
		(0.1)		(0.1)	(0)	(0)	(0.1)
Glc_5[a]	Internal residues	103.2	73.8	74.8	79.1	75.7	60.7
Glc_5-monoAc		101.3	73.9	74.9	79.2	75.7	60.8
		(−1.9)	(0.1)	(0.1)	(0.1)	(0)	(0.1)
Glc_5[a]	Non-reducing end residue	103.4	74.0	76.3	70.3	76.8	61.4
Glc_5-monoAc		101.3	72.4	77.9	68.6	76.6	61.3
		(−2.1)	(−1.6)	(+1.6)	(−1.7)	(−0.2)	(−0.1)

The values in parentheses are the chemical shifts differences to the β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (Glc_5). Positive differences indicate the upfield shifts.
[a] Data from Ref. [31]. Chemical shifts at C-2 (−1.6), C-3 (+1.6) and C-4 (−1.7) are in agreement with those (−1.7, +1.8, −1.7) reported in Ref. [29].

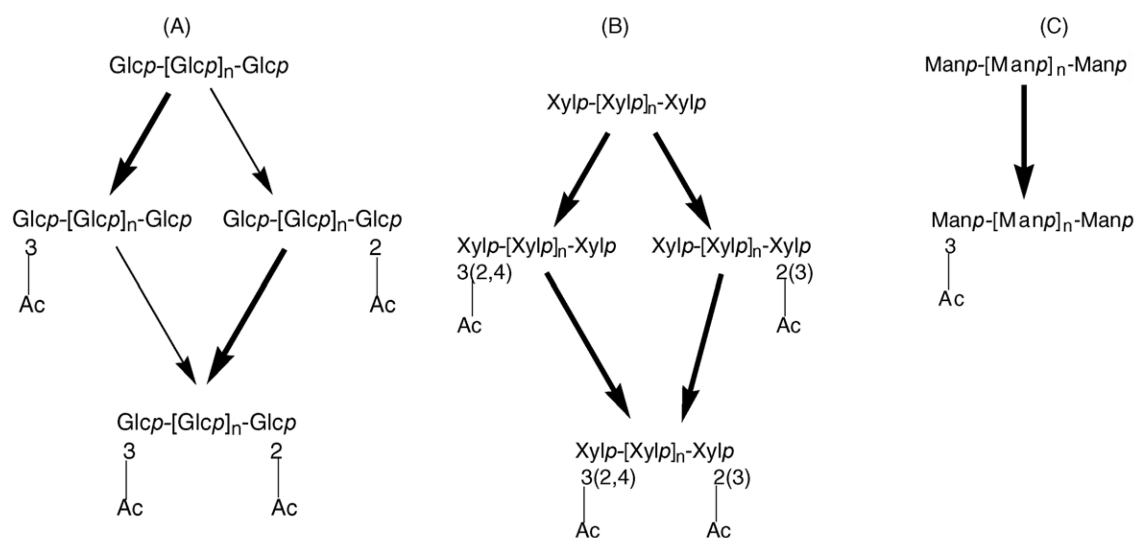

Fig. 4. Scheme of acetylation of (A) cello-, (B) xylo- and (C) manno-oligosaccharides by *T. reesei* acetyl esterase in water–vinyl acetate two-phase system. Thick arrows indicate faster acetylations and thin arrows slower acetylations.

lated at *O*-3 position of the non-reducing end mannopyranosyl unit.

The positions of acetyl groups in xylooligosaccharides were not determined due to the formation of multiple derivatives of mono- and di-acetates of xylooligosaccharides, most probably as a result of a spontaneous acetyl group migration along the flexible xylopyranose ring [24]. The rate of acetyl group migration in water is significant and disables the isolation and identification of the primary reaction products under the used experimental conditions. However, in terms of the similarity in the spatial structure of gluco- and xylo-oligosaccharides, one can assume that the enzyme acetylates xylooligosaccharides also at position *O*-3 of the non-reducing xylopyranosyl residue and at *O*-2 position of the reducing end xylose.

4. Conclusion

Trichoderma reesei acetyl esterase catalyzes acetylation at *O*-3 position of terminal units of gluco-, xylo- and manno-oligosaccharides and *O*-2 position at reducing end units of gluco- and xylo-oligosaccharides (Fig. 4). Axial hydroxyl group at position 2 of reducing end mannose in mannooligosaccharides is not recognized by the enzyme and its acetylation, as the second acetylation, was not observed. The major role of this unique *T. reesei* acetyl esterase is to liberate acetyl groups from terminal units of oligosaccharides and make them accessible for further hydrolysis catalyzed by glycosidases, thus it can be classified as an exo-acting acetyl esterase. The unique transacetylation catalyzed by the enzyme in aqueous media, represents not only a new environmentally friendly way of efficient regioselective acetylation of oligosaccharides, but also an attractive option of modification of terminal carbohydrate residues of variety of natural glycosides, such as glycosides of flavonoids, antibiotics and terpenoids. The reaction could be significant for reduction of the hydrophilic character of some of these biologically important molecules, increasing thus their compatibility with biological membranes.

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